

*Sub A*  
*b1*

**What is claimed is:**

1 A method for classifying and counting leukocytes comprising the steps of:

5 (1) adding to a hematological sample the following fluorescence-labeled antibodies labeled with fluorescent dyes which emit fluorescences distinguishable from each other;

10 (a) a first fluorescence-labeled antibody which bonds specifically to leukocytes,

(b) a second fluorescence-labeled antibody which bonds to at least one kind of neutrophilic cells, and

15 (c) a third fluorescence-labeled antibody which bonds to at least one kind of immature granulocytic cells, in order to stain leukocytic cells in the hematological sample, and

20 removing erythrocytes from the hematological sample;

(2) analyzing the resulting hematological sample using a flow cytometer to measure at least one scattered light signal and three separate fluorescence signals;

25 (3) defining a group of granulocytic cells on the basis of intensity of the scattered light and intensity of fluorescence from the first fluorescence-labeled antibody;

(4) defining neutrophilic cells in the defined group of granulocytic cells on the basis of the intensity of the fluorescence from the first fluorescence-labeled antibody and intensity of fluorescence from the second or third fluorescence-labeled antibody;

*b1*

~~(5) classifying the defined group of the neutrophilic cells into groups of neutrophilic cells different in degree of maturity on the basis of the intensity of the fluorescence from the second fluorescence-labeled antibody and the intensity of the fluorescence~~

5 ~~from the third fluorescence-labeled antibody, and~~

~~counting the number of cells in each of the groups.~~

2. ~~A method according to claim 1, wherein in step (3), a group of all leukocytic cells is defined and counted on the basis of the intensity of the scattered light and the intensity of the fluorescence from the first fluorescence-labeled antibody in addition to the group of granulocytic cells, and in step (5), the ratio of the number of the neutrophilic cells in each of the groups different in degree of maturity with respect to the number of all the leukocytic cells is calculated.~~

15 3. ~~A method according to claim 1, wherein the first fluorescence-labeled antibody comprises an anti-CD45 antibody.~~

4. ~~A method according to claim 1, wherein the second fluorescence-labeled antibody comprises an antibody selected from the group consisting of an anti-CD11b antibody, an anti-CD16 antibody, an anti-CD66b antibody and an anti-CD66c antibody, and the third fluorescence-labeled antibody comprises an antibody selected from the same group but different from the antibody of the second fluorescence-labeled antibody.~~

20 5. ~~A method according to claim 1, wherein the second and third fluorescence-labeled antibodies comprises any combination of~~

an anti-CD16 antibody with an anti-CD11b antibody, an anti-CD16 antibody with an anti-CD66b antibody, an anti-CD16 antibody with an anti-CD66c antibody, an anti-CD11b antibody with an anti-CD66b antibody, and an anti-CD11b antibody with an anti-CD66c antibody.

5 antibody.

6. A method according to claim 5, wherein the second and third fluorescence-labeled antibodies comprise the anti-CD16 antibody and the anti-CD11b antibody.

7. A method according to claim 1, wherein the scattered light measured is side scattered light.

8. A method according to claim 1, wherein the fluorescent dyes are selected from the group consisting of fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), Texas Red, PE-CY5 and peridinin chlorophyll protein (PerCP).

15 9. A method according to claim 7, wherein the fluorescent dyes of the first, second and third fluorescence-labeled antibodies for emitting distinguishable fluorescences comprise a combination of FITC, PE and PE-CY5 or a combination of FITC, PE and PerCP.

10. A method according to claim 1, wherein the hematological 20 sample is a sample of peripheral blood, bone marrow fluid or urine of a mammal or a sample collected from a mammal by apheresis.

11. A method according to claim 1, wherein in step (1), the leukocytic cells are fluorescence-stained after erythrocytes are removed from the hematological sample.

add  
b2

add  
D